Week 1 (6/6 – 6/10)

Analyzed Sanger sequence results for synthetic β' recombinase coding DNA sequences (CDS) from IDT. Some gBLOCKs (11, 13, 3, 4) were found to contain errors in the start codon (ATG → TTG) and some internal nonsilent SNP's. Others contained no significant errors, and were therefore sequence-validated (gBLOCKs 1, 2, 14, 15). Learned how to perform MAGE in E. coli from Jaymin. Discussed recombinase sequences and making competent cells with Dr. Moreno. Obtained constructs stocks. Helped take inventory of the lab.

Table of synthetic β' recombinase coding DNA gBLOCKs. Sequences available upon request.

gBLOCK ID	Accession Number	Type	Phage Host
--	**P03698**	**λ-red β protein**	**E. coli**
1	A6UAS9_SINMW	Phage recombination protein Bet	S. medicae WSM419
2	B5ZVM8_RHILW	Phage recombination protein Bet	R . leguminosarum WSM2304
3	H0FXW6_RHIML	Phage recombination protein Bet	S. meliloti RM41
4	K0PZW0_9RHIZ	Phage recombination protein Bet	R. mesoamericanum STM3625
11	D3_ERF_Q9MC70	Bacteriophage recombination protein	Bacillus Phage SPP1
12	J0GZ13_RHILW	Bacteriophage recombination protein	R. leguminosarum bv. trifolii WSM597
13	N6VDJ4_9RHIZ	Bacteriophage recombination protein	R. freirei PRF81
14	SPP1_GP35_Q38143	Bacteriophage recombination protein	Bacillus Phage SPP1
15	F7X3D1_SINMM	Bacteriophage recombination protein	S. meliloti SM11

There were irreconcilable issues with synthesis of gBLOCK 12, so it was not used in experimentation.

Week 2 (6/13 – 17)

Scanned literature for ideas about desiccation resistance for applications in terraforming Mars. Identified trehalose OtsAB pathway, EPS biosynthetic pathways, and tardigrade cryptobiotic state were identified as jumping-off points.

Designed with Leslie primers for a sequencing reaction to validate citrine CDS sequence and integration into the LIC cassette of iProm or cProm pZP200-Del* expression vectors created earlier (Natalie Ma, Maria Moreno, iGEM team and class participants in MCDB 201L), ie translation-validate the constructs.

Leslie planned to test the promoter activity with a fluorescence assay later on, so we wanted exclude the possibility that mutations in the citrine CDS or improper or no integration into the cassette causing a negative fluorescence assay read.

Once primers came in, prepared sequencing reaction and sent off to GeneWhiz.

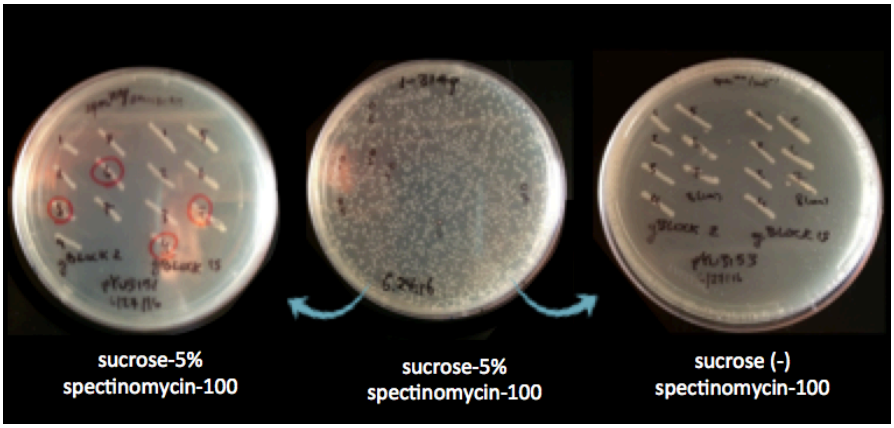
Week 3 (6/20 – 24)

Validated β' recombinase CDS gBLOCKs (IDT, gB 1, 2, 14, and 15) were amplified and LIC-extended by using primers that added end-specific stuffer sequence for T4 exonuclease to create complementary overhangs appropriate to directional integration via LIC. These overhangs were homologous to those found in the LIC cassette of expression plasmids (pZP00-Del*) after treatment and digestion with BsaI and T4 vectors.

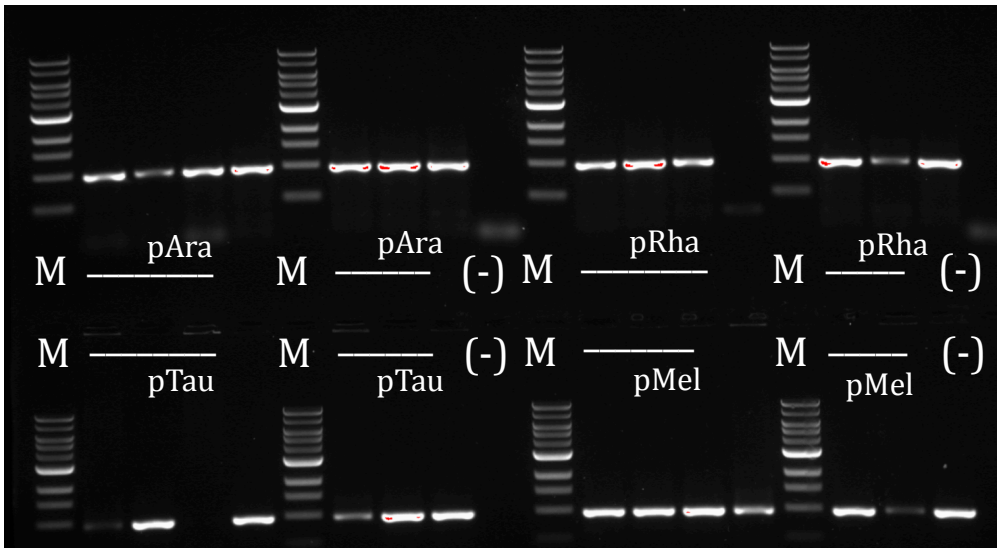
4 of the 16 gBLOCKs (gB 1, 2, 14 and 15) were identified in week 1 as having no nonsilent SNP's, so these were the 4 gBLOCKs that were extended for LIC'ing into iProm expression vectors. Leslie identified issues with the constitutive promoter RBS's, reactions were planned to integrate only into iProm until the RBS issue was resolved.

Week 4 (6/27 – 7/1)

Ligation independent cloning was performed with these extended gBLOCKs. Four gBLOCKs were integrated into four different kinds of rhizobia- specific inducible promoter (arabinose, melliboise, taurine, and rhamnose , Mostfavsi et al., 2014) meaning 16 different iProm-gBLOCK (beta homolog type) variants total. After cloning, they were transformed into *E. coli* (Invitrogen TOP 10 One Shot). Cells were grown on Spectinomycin-100 and sucrose-5% to select for recombinants with the antibiotic resistance gene of pZP200-Del* shuttle vector and replacement of the SacB sucrose lethality gene with β' recombinase gBLOCK.



Colony PCR was performed on each variant (7 colonies selected from each plate) to see if the cells contained the gBLOCK sequence. Product was amplified with the same primers used to LIC-extend the recombinase gBLOCKs in week 3. Expected product (~1 kB long) was found in 105 out of 112 colonies, with at least 5 colonies per variant displaying expected band per iProm-recombinase variant.



Gel results for gBLOCK 15 confirming plasmid and insert in transformed cells.

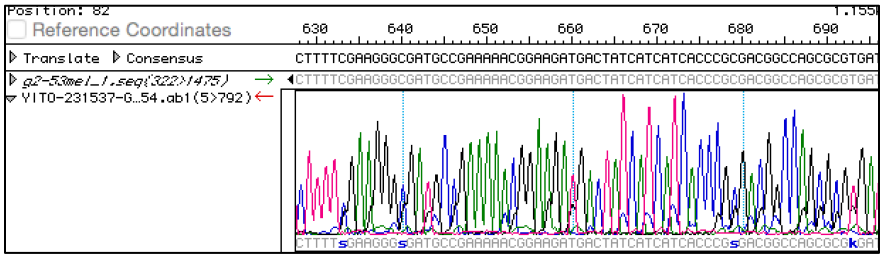
pDNA was isolated from two colonies per variant using QIAGEN miniprep. Designed with Zach primers to generate a 400 bp fragment for each iProm- β' recombinase combination and synthesized. Sanger sequencing reactions were prepared with this pDNA and aforementioned primers to verify that the integration of recombinase into the pZP200-Del* LIC site was correct and ideal for expression (eg translational).

Week 5 (7/4 – 8)

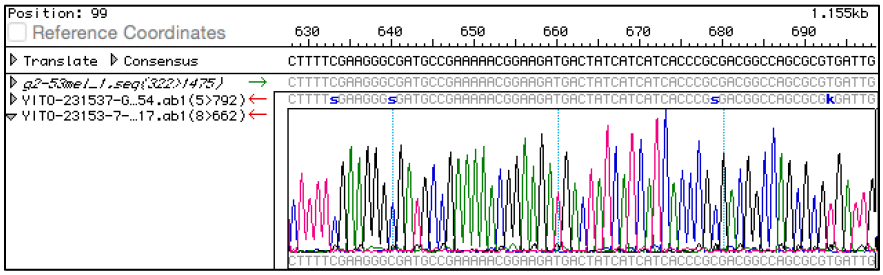
The last of the sequencing reactions for the 16 iProm- β' recombinase variants were sent off. gBLOCKs 11 and 13, that had been found in an earlier analysis (Week 1) to contain nonsilent errors from synthesis, were rescreened via cloning in TOPO vectors (Invitrogen), selection on kanamycin, and extraction/sequencing to find that two of the clones (11-1 and 13-2) did not contain any significant errors.

gBLOCks containing one nonsilent SNP (3 and 4) were amplified from the 5' or 3' end to the SNP to create two putatively corrected fragments of corrected sequence per gBLOCK.
via crossover PCR in an attempt to correct the SNP. (gBLOCKs 3 and 4).

Returned sequence results for gBLOCKs 1, 2, 14 and 15 suggested correct translational fusion of gBLOCKs to promoter and terminator in LIC cassette, ie these variants were translation-validated.



pMel (pYU3153), gBLOCK 15, Clone 7 (+)



pMel (pYU3153), gBLOCK 15, Clone 7 (-)

Sequence results for gBLOCK 15 confirming translational fusion (translation validation) .

Week 6 (7/11 – 15)

The two newly sequence-validated gBLOCKs from 11-1 and 13-2 were LIC-extended and inserted into the four different iProm vectors using LIC, creating eight additional iProm- β' recombinase variants. Top 10 *E. coli* cells were transformed with the iProm-gB11 and iProm-13 expression vectors. Cells were plated on Spectinomycin-100 and sucrose 5% for antibiotic selection. Colony PCR was performed on seven colonies from each variant to determine if cells contained plasmid with insert. Expected PCR product length was observed for both gBLOCKs.

The two fragments for gBLOCKs 3 and 4 were annealed in a crossover PCR reaction to generate either LIC-extended or TOPO-vector backbone ends, depending on use of generic TOPO sequencing primers or LIC extension primers to amplify and anneal in the second reaction. Two promising lanes from the resultant product were combined and cloned into TOPO-II zero blunt vectors (Invitrogen). pDNA was extracted and amplified, with gel results exhibiting expected product length for gBLOCK 3 but not 4. Additional clones of 4 were amplified, with variable lengths identified in gel. Two promising lanes were chosen from each for sequencing.

S. meliloti 1021 and R. tropici CIAT 899 containing pORTMAGE-3 were electroporated and transformed (Biorad *Agrobacterium* protocol) with the

validated iProm- β' recombinase variants (iProm- gB 1, 2, 14 and 15) to create 16 strains per type (32 in all).

Week 7 (7/18 – 22)

Sequence reactions for extracted pDNA from zero blunt vectors containing gB 3 and 4 were prepared to determine if modified crossover PCR site directed mutagenesis was successful.

Rhizobia cells transformed with translation-validated constructs and pORTMAGE-3 were plated on kanamycin-50 (for pORTMAGE-3 abR gene) and spectinomycin-100 (for expression vector pZP200-Del* abR gene) for antibiotic selection. Seven colonies from each variant were then selected for colony PCR to determine if the cells contained both pORTMAGE-3 and pZP- β' expression vectors.

Sequencing reactions were prepared for β' gBLOCKs 11 and 13 to determine if the β' recombinase CDS fusion into the LIC expression cassette was translational using primers designed to generate 400 bp product.

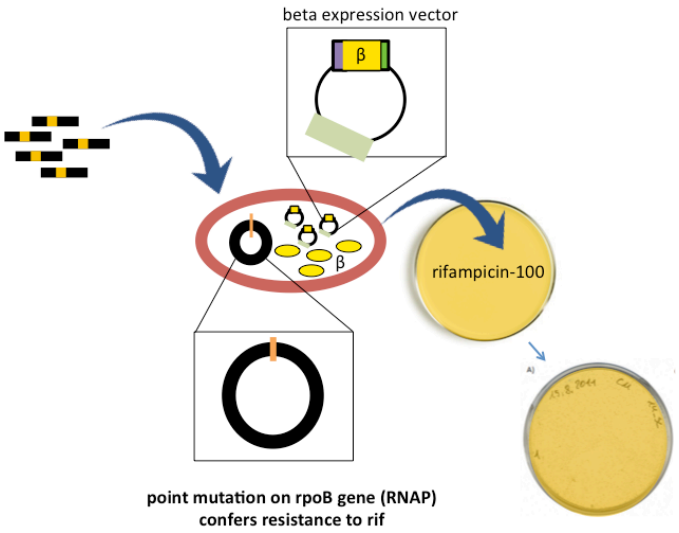
Week 8 (7/25 – 29)

Sequence results from zero blunt vectors containing gB 3 and 4 indicated modified crossover PCR site directed mutagenesis for these recombinase CDS's was unsuccessful.

Sequencing results from β' gBLOCKs 11 and 13 indicated β' recombinase CDS fusion into the LIC expression cassette was ambiguously translational. Some clones of gB 13 appeared to have no nonsilent SNPs, while most clones of iProm-gB 11 contained multiple errors. Future experimentation required.

For *Rhizobia* cells transformed with translation validated iProm- β' expression vectors and growing on antibiotic selection plates (two variants did not grow, more cells were transformed with these vectors), colony PCR was performed with seven colonies from each variant. No bands were observed for *R. tropici* CIAT 899 cells, consistent with other lab member's (Miny and Gloria's) results, suggesting that the *R. tropici* PCR protocol was in need of optimization. Some colonies for *S. meliloti* displayed both the expected 600 bp band for pORTMAGE-3 and 400 bp band for pZP β' homolog expression vector.

Glycerol stocks were prepared from *S. meliloti* cells containing both plasmids. These strains are potentially ready for MAGE.



Rifampicin assay for MAGE efficiency

Week 10+

With Leslie, transformed *E. coli* (Top 10 One Shot) with Lionel's pTac and Anderson Medium from last year. Under UV light, these cells were observed to fluoresce. With Leslie and Natalie, screened potential biobricks for illegal restriction sites and designed primers for amplifying biobricks from plasmids with Leslie.

Conducted Gloria's modified *R. tropici* PCR protocol with lyse step on transformed CIAT 899 cells, without positive results.